

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year)
 17 July 2000 (17.07.00)

Applicant's or agent's file reference
 44.17.67505/001

International application No.
 PCT/GB99/03756

Priority date (day/month/year)
 11 November 1998 (11.11.98)

International filing date (day/month/year)
 11 November 1999 (11.11.99)

Applicant

DØSKELAND, Stein, Ove et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

 09 June 2000 (09.06.00)

in a notice effecting later election filed with the International Bureau on:

2. The election was
 was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Juan Cruz

Telephone No.: (41-22) 338.83.38

GB9903756

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

COCKBAIN, Julian
 Frank B. Dehn & Co.
 179 Queen Victoria Street
 London EC4V 4EL
 ROYAUME-UNI

Date of mailing (day/month/year)
 25 September 2000 (25.09.00)

Applicant's or agent's file reference
 44.17.67505/001

IMPORTANT NOTIFICATION

International application No.
 PCT/GB99/03756

International filing date (day/month/year)
 11 November 1999 (11.11.99)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address

COCKBAIN, Julian
 102 Cranbrook Road
 London W4 2LJ
 United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address

BIOSENSE LABORATORIES AS
 HIB-Thormøhlensgate 55
 N-5008 Bergen
 Norway

State of Nationality

NO

State of Residence

NO

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

The applicant in Box 1 has assigned all rights to the applicant indicated in Box 2.

4. A copy of this notification has been sent to:

 the receiving Office the designated Offices concerned the International Searching Authority the elected Offices concerned the International Preliminary Examining Authority other:

The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland

Authorized officer

Aino Metcalfe

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

COCKBAIN, Julian
 Frank B. Dehn & Co.
 179 Queen Victoria Street
 London EC4V 4EL
 ROYAUME-UNI

Date of mailing (day/month/year)
 25 September 2000 (25.09.00)

Applicant's or agent's file reference
 44.17.67505/001

IMPORTANT NOTIFICATION

International application No.
 PCT/GB99/03756

International filing date (day/month/year)
 11 November 1999 (11.11.99)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address
 DØSKELAND, Stein, Ove
 SERRES, Margrethe, Hauge
 FLADMARK, Kari, Espolin

State of Nationality	State of Residence
NO	NO
Telephone No.	
Facsimile No.	
Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address
 BIOSENSE LABORATORIES AS
 HIB-Thormøhlensgate 55
 N-5008 Bergen
 Norway

State of Nationality	State of Residence
NO	NO
Telephone No.	
Facsimile No.	
Teleprinter No.	

3. Further observations, if necessary:

The applicant/inventors in Box 1 have assigned their rights for all designated States
 except US to the new applicant indicated in Box 2.

4. A copy of this notification has been sent to:

the receiving Office
 the International Searching Authority
 the International Preliminary Examining Authority

the designated Offices concerned
 the elected Offices concerned
 other:

The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland
 Facsimile No.: (41-22) 740.14.35

Authorized officer

Aino Metcalfe

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

COCKBAIN, Julian
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
GRANDE BRETAGNE

PCT

67505 / 99

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

12.12.2000

Applicant's or agent's file reference
44.17.67505/001

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03756

International filing date (day/month/year)
11/11/1999

Priority date (day/month/year)
11/11/1998

Applicant
BIOSENSE LABORATORIES AS et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Digiusto, M

Tel. +49 89 2399-8162



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 44.17.67505/001	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB99/03756	International filing date (day/month/year) 11/11/1999	Priority date (day/month/year) 11/11/1998
International Patent Classification (IPC) or national classification and IPC G01N33/53		
Applicant BIOSENSE LABORATORIES AS et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 6 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application 		

Date of submission of the demand 09/06/2000	Date of completion of this report 12.12.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Moreno de Vega, C Telephone No. +49 89 2399 7486



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03756

I. Basis of the report

1. This report has been drawn on the basis of (substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):

Description, pages:

1,2,4-24 as originally filed

3,3a as received on 03/11/2000 with letter of 01/11/2000

Claims, No.:

21 as originally filed

1-20 as received on 03/11/2000 with letter of 01/11/2000

Drawings, sheets:

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03756

4. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-14, 17-20
	No:	Claims 15, 16
Inventive step (IS)	Yes:	Claims 1-14, 18-20
	No:	Claims 15-17

Industrial applicability (IA) Yes: Claims 1-20
 No: Claims

2. Citations and explanations
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03756

Reference is made to the following documents:

D1: US-A-5 180 665 (HOLMES CHARLES) 19 January 1993 (1993-01-19)
D2: EP-A-0 554 458 (IATRON LAB ;OSAKA PREFECTURE (JP)) 11 August
1993 (1993-08-11)

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. D1 discloses a method for quantitatively assaying the presence in marine samples of diarrheic shellfish poisoning (DSP) toxins having phosphatase inhibitory activity comprising preparing and fractionating a marine extract, incorporating at least one protein phosphatase inhibited by said toxin and a labelled physiological substrate and measuring the amount of toxin present in said extract by means of said label and said substrate. This document does not disclose a method using a solid support having an immobilized ligand.

D2 (see claims 4-6, page 7 lines 7-43) discloses a method of determination of DSP toxins in which an organic solvent extract of the sample is contacted with a first antibody to the toxin to be determined immobilized on an insoluble carrier, adding an excess amount of a labelled second antibody which binds to the toxin and measuring signals from said label on said second antibody.

1. Novelty (Article 33(2) PCT)

Claims 1-14 and 17-20 are considered to be new, because the method for determining phosphatase targeting toxins which inhibit protein phosphatases comprising contacting a solid support having a ligand immobilized thereon with a sample and a non-immobilized ligand, wherein the toxin binding ligand is a protein phosphatase enzyme, is not disclosed in the known prior art. Thus, claims 1-14 and 17-20 meet the requirements of Article 33(2) PCT.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03756

Claims 15 and 16 are not considered to be new, because kits comprising solid phases on which a ligand is immobilized, a non immobilized ligand being a protein phosphatase enzyme, added for producing fluorescence or a change in colour upon cleaving an adequate substrate, and a report moiety are already known in immunoassays.

Therefore, claims 15 and 16 do not meet the requirements of Article 33(2) PCT.

2. Inventive step (Article 33(3) PCT).

Dependent claim 17 does not contain any features which, in combination with the features of any claim to which it refers, meet the requirements of the PCT in respect of inventive step in the light of the general knowledge in the field.

D2, which is considered to be the most relevant prior art, differs from the present invention in that in the method for determining DSP toxins the toxin binding ligand is an antibody and not a protein phosphatase enzyme, and in that D2 does not disclose the kit and its use of claims 18-20. The technical problem to be solved by the present invention is the provision of an improved assay for the qualitative and quantitative determination of phosphatase-targeting toxins and kits therefor, which can be performed by non-skilled personnel without requiring laboratory equipment. There is no hint in D1 and D2 to combine their teachings arriving at the method and kits of present claims 1-4, 18-20, which therefore meet the requirements of Article 33(3) PCT.

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
COCKBAIN, Julian
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
GRANDE BRETAGNE

675-5/001

PCT

WRITTEN OPINION

(PCT Rule 66)

Applicant's or agent's file reference 44.17.67505/001		Date of mailing (day/month/year) 28.07.2000
International application No. PCT/GB99/03756	International filing date (day/month/year) 11/11/1999	Priority date (day/month/year) 11/11/1998
International Patent Classification (IPC) or both national classification and IPC G01N33/53		
Applicant D SKELAND, Stein, Ove et al.		

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I Basis of the opinion
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain document cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

The final date by which the international preliminary examination report must be established according to Rule 69.2 is: **11/03/2001**.

Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer / Examiner Moreno de Vega, C Formalities officer (incl. extension of time limits) Digiusto, M Telephone No. +49 89 2399 8162
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I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-24 as originally filed

Claims, No.:

1-21 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims 1-5, 7, 10, 11, 14, 16, 17
Inventive step (IS)	Claims 1-7, 10, 11, 14-18
Industrial applicability (IA)	Claims

2. Citations and explanations

s e separat sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: US-A-5 180 665 (HOLMES CHARLES) 19 January 1993 (1993-01-19)
D2: EP-A-0 554 458 (IATRON LAB; OSAKA PREFECTURE (JP)) 11 August
1993 (1993-08-11)

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. D1 discloses a method for quantitatively assaying the presence in marine samples of diarrheic shellfish poisoning (DSP) toxins having phosphatase inhibitory activity comprising preparing and fractionating a marine extract, incorporating at least one protein phosphatase inhibited by said toxin and a labelled physiological substrate and measuring the amount of toxin present in said extract by means of said label and said substrate. This document does not disclose a method using a solid support having an immobilized ligand.

D2 (see claims 4-6, page 7 lines 7-43) discloses a method of determination of DSP toxins in which an organic solvent extract of the sample is contacted with a first antibody to the toxin to be determined immobilized on an insoluble carrier, adding an excess amount of a labelled second antibody which binds with the toxin and measuring signals from said label on said second antibody. This document appears to be novelty destroying for claims 1-5, 7, 10, 11 and 14.

Due to the broad and unspecific terms of claims 16 and 17, these are not considered to be new, because kits comprising solid phases on which a ligand is immobilized, a non immobilized ligand and a reporter moiety are of common use and well known in immunoassays.

Thus, claims 1-5, 7, 10, 11, 14, 16 and 17 do not comply with the requirements of Article 33(2) PCT.

2. Claims 6, 15 and 18 do not contain any additional technical feature which could be considered to involve an inventive step in the light of the disclosure in D2. Thus, claims 6, 15 and 18 do not meet the requirements of Article 33(3) PCT.
3. Claims 8, 9, 12, 13, 19, 20 and 21, considering its reference to claims 19-20, are considered to be new, because the method for determining phosphatase targeting toxins and a kit therefore as featured in these claims has not been disclosed in the prior art. D2, which is considered to be the most relevant prior art, differs from present claims 8, 9, 12, 13, 19, 20 and 21 in that in the method for determining DSP toxins the toxin binding ligand is an antibody and not a protein phosphatase enzyme or a labelled peptide hepatotoxin or labelled okadaic acid, and in that D2 does not disclose the kit and its use of claims 19-21. The technical problem to be solved by the present invention is the provision of an improved assay for the qualitative and quantitative determination of phosphatase-targeting toxins and kits therefor. There is no hint in the prior art to arrive at the solution proposed by claims 8, 9, 12, 13, 19-21, which therefore meet the requirements of Article 33(3) PCT.

Re Item VII

Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

1. Claims 16 and 18 do not meet the requirements of Article 6 PCT, because the wording "a signal readable without laboratory equipment" is unclear.
2. The reference "according to the invention" in claim 16 is not clear (Article 6 PCT). Reference should be made to the respective claims.

REC'D 15 DEC 2000

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 44.17.67505/001	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB99/03756	International filing date (day/month/year) 11/11/1999	Priority date (day/month/year) 11/11/1998
International Patent Classification (IPC) or national classification and IPC G01N33/53		
Applicant BIOSENSE LABORATORIES AS et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 6 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application 		

Date of submission of the demand 09/06/2000	Date of completion of this report 12.12.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Moreno de Vega, C Telephone No. +49 89 2399 7486



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03756

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*):

Description, pages:

1,2,4-24	as originally filed		
3,3a	as received on	03/11/2000 with letter of	01/11/2000

Claims, No.:

21	as originally filed		
1-20	as received on	03/11/2000 with letter of	01/11/2000

Drawings, sheets:

1/4-4/4	as originally filed
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2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03756

4. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-14, 17-20
	No:	Claims 15, 16
Inventive step (IS)	Yes:	Claims 1-14, 18-20
	No:	Claims 15-17
Industrial applicability (IA)	Yes:	Claims 1-20
	No:	Claims

2. Citations and explanations
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03756

Reference is made to the following documents:

D1: US-A-5 180 665 (HOLMES CHARLES) 19 January 1993 (1993-01-19)
D2: EP-A-0 554 458 (IATRON LAB ;OSAKA PREFECTURE (JP)) 11 August
1993 (1993-08-11)

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. D1 discloses a method for quantitatively assaying the presence in marine samples of diarrheic shellfish poisoning (DSP) toxins having phosphatase inhibitory activity comprising preparing and fractionating a marine extract, incorporating at least one protein phosphatase inhibited by said toxin and a labelled physiological substrate and measuring the amount of toxin present in said extract by means of said label and said substrate. This document does not disclose a method using a solid support having an immobilized ligand.

D2 (see claims 4-6, page 7 lines 7-43) discloses a method of determination of DSP toxins in which an organic solvent extract of the sample is contacted with a first antibody to the toxin to be determined immobilized on an insoluble carrier, adding an excess amount of a labelled second antibody which binds to the toxin and measuring signals from said label on said second antibody.

1. Novelty (Article 33(2) PCT)

Claims 1-14 and 17-20 are considered to be new, because the method for determining phosphatase targeting toxins which inhibit protein phosphatases comprising contacting a solid support having a ligand immobilized thereon with a sample and a non-immobilized ligand, wherein the toxin binding ligand is a protein phosphatase enzyme, is not disclosed in the known prior art. Thus, claims 1-14 and 17-20 meet the requirements of Article 33(2) PCT.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03756

Claims 15 and 16 are not considered to be new, because kits comprising solid phases on which a ligand is immobilized, a non immobilized ligand being a protein phosphatase enzyme, added for producing fluorescence or a change in colour upon cleaving an adequate substrate, and a report moiety are already known in immunoassays.

Therefore, claims 15 and 16 do not meet the requirements of Article 33(2) PCT.

2. Inventive step (Article 33(3) PCT).

Dependent claim 17 does not contain any features which, in combination with the features of any claim to which it refers, meet the requirements of the PCT in respect of inventive step in the light of the general knowledge in the field.

D2, which is considered to be the most relevant prior art, differs from the present invention in that in the method for determining DSP toxins the toxin binding ligand is an antibody and not a protein phosphatase enzyme, and in that D2 does not disclose the kit and its use of claims 18-20. The technical problem to be solved by the present invention is the provision of an improved assay for the qualitative and quantitative determination of phosphatase-targeting toxins and kits therefor, which can be performed by non-skilled personnel without requiring laboratory equipment. There is no hint in D1 and D2 to combine their teachings arriving at the method and kits of present claims 1-4, 18-20, which therefore meet the requirements of Article 33(3) PCT.

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algal toxins, as mentioned above the phosphatase targeting toxins microcystin and nodularin have been found to be tumour promoters and it is believed that repeated exposure to such toxins at the clinical or sub-clinical level, particularly in combination with a high intake of alcohol or smoking may result in cancer, especially of the liver.

Presently, a number of different methods exist for the detection and quantitation of phosphatase targeting toxins, from algae and cyanobacteria. One standard method involves grinding mussels or other potential sources of the phosphatase targeting toxins and injecting an extract of the ground mussel tissue into mice. The presence and level of phosphatase-targeting toxin contamination is then determined in relation to mouse survival (Stabell et al. (1992), Food. Chem. Toxicol. 30(2): 139-44). Clearly, this is a time consuming, crude and expensive method of assessing food safety and quality control.

Another method for determining diarrheal shellfish poisons (DSP) (EP-A-554458 of Iatron Laboratories Inc) involves the use of a first and second antibody to the toxin in a conventional sandwich assay.

Another method involves measuring the reduction in enzymic activity of exogenously added phosphatase thus detecting the presence of phosphatase targeting toxins in the shellfish. Again this involves grinding mussels or other shellfish tissue, releasing endogenous phosphatases which interfere with the added phosphatase, compromising the sensitivity and accuracy of the test (Sim and Mudge (1994) in Detection Methods for Cyanobacterial Toxins Eds. Codd, Jeffries, Keevil and Potter, Royal Society of Chemistry, and US-A-5180665 of Charles Holmes).

A great need exists therefore for a quick, sensitive, and inexpensive assay or method to allow the qualitative and/or quantitative determination of the

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presence of phosphatase-targeting toxins, in particular
algal and cyanobacterial phosphatase-targeting toxins,
in water, shellfish and/or edible products of algae or
cyanobacteria. In particular, there is a need for an
assay method which is simple enough to be performed on
5

- 25 -

Claims:

1. An assay method for determining phosphatase targeting toxins which inhibit protein phosphatases comprising contacting a solid support having an immobilized ligand immobilized thereon with:

(i) a sample suspected of being contaminated with toxin and

(ii) a non-immobilized ligand,

wherein said immobilized ligand is capable of binding to at least one of said toxins, to said non-immobilized ligand or to complexes of said toxin and said non-immobilized ligand, and said non-immobilized ligand is capable of binding to at least one of said immobilized ligand, to said toxin or to complexes of said toxin and said immobilized ligand whereby the proportion of said immobilized ligand bound by said toxin, said non-immobilized ligand or complexes of said toxin and said non-immobilized ligand is dependent on the toxin content of said sample and wherein the immobilized and/or non-immobilized toxin binding ligand is a protein phosphatase enzyme and

wherein said immobilized ligand is capable of generating directly or indirectly detectable signal when uncomplexed, when complexed by said toxin, when complexed by a complex of said toxin and said non-immobilized ligand or when complexed by said non-immobilized ligand or said non-immobilized ligand is capable of generating a directly or indirectly detectable signal when uncomplexed or when complexed, separating a bound fraction from a non-bound fraction; and

directly or indirectly determining the non-immobilized ligand bound to the immobilized ligand (the bound fraction) or non-complexed in aqueous solution (the non-bound fraction);

wherein the application of (i) and (ii) to the

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solid support may be performed separately, sequentially or simultaneously and if separately or sequentially, they can be performed in either order.

5 2. An assay method as claimed in claim 1 wherein said phosphatase-targeting toxin is produced by algae or by cyanobacteria.

10 3. An assay method as claimed in either of claims 1 and 2 wherein the toxin to be determined is a heptatoxin or okadaic acid.

15 4. An assay method as claimed in any one of claims 1 to 3 wherein toxin molecules present in the sample compete with the non-immobilized ligand for a limited number of binding sites of the immobilized ligand and any toxin present in said sample is determined relative to the extent of non-immobilized ligand bound to or not bound to the binding sites of the immobilized ligand.

20 5. An assay method as claimed in any one of claims 1 to 4 wherein the presence or absence of a phosphatase-targeting toxin is determined.

25 6. An assay method as claimed in any one of claims 1 to 5 wherein the sample under investigation is surface or free moisture from shellfish, or water taken from the habitat in which such shellfish live, or water taken from domestic water supplies.

30 7. An assay method as claimed in any one of claims 1 to 6 wherein the immobilized or non-immobilized ligand is an antibody or antibody fragment.

35 8. An assay method as claimed in any one of claims 1 to 7 wherein the protein phosphatase enzyme is the binding ligand protein phosphatase 2A.

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9. An assay method as claimed in any one of claims 1 to 8 wherein either the immobilised or non-immobilised ligands carries a reporter moiety.

5 10. An assay method as claimed in claim 9 wherein the non-immobilized ligand carries a reporter moiety.

11. An assay method as claimed in claim 10 wherein the
10 non-immobilized ligand is a labelled peptide hepatotoxin or labelled okadaic acid.

12. An assay method as claimed in claim 11 wherein the hepatotoxin is selected from nodularin, microcystin LC or microcystin YR.

15 13. An assay method as claimed in any one of claims 1 to 12 wherein the solid support is a dipstick or solid matrix.

20 14. An assay method as claimed in claim 13 wherein the solid matrix is polymeric or magnetic beads.

15. A kit for the detection of phosphatase-targeting toxins which inhibit protein phosphatases, said kit comprising:
25 a solid phase upon which is immobilised a ligand;
a non-immobilized ligand, preferably in aqueous solution or complexed to the immobilized ligand;
and wherein the immobilized and/or non-immobilized ligand is a protein phosphatase enzyme;
30 where neither of said immobilized and non-immobilized ligands includes a directly or indirectly detectable moiety, a reporter moiety capable of binding to one of said immobilized and non-immobilized ligands and generating a detectable signal, preferably said detectable moiety or signal being directly readable without laboratory equipment.

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16. A kit as claimed in claim 15 wherein said phosphatase-targeting toxin is produced by algae or by cyanobacteria.

5 17. A kit as claimed in either of claims 15 and 16 wherein said kit comprises:
a solid phase upon which is immobilized a protein phosphatase enzyme as a toxin binding ligand;
a reporter molecule capable of competitively
10 inhibiting binding of phosphatase-targetting toxins to said toxin binding ligand and generating a signal readable without laboratory equipment.

15 18. A kit as claimed in any one of claims 15 to 17 wherein said kit comprises magnetically displaceable polymer micro spheres having immobilized thereon a protein phosphatase;
gold sol labelled peptide hepatotoxin molecules capable of competitively inhibiting cyanobacterial
20 toxins binding to said protein phosphatase.

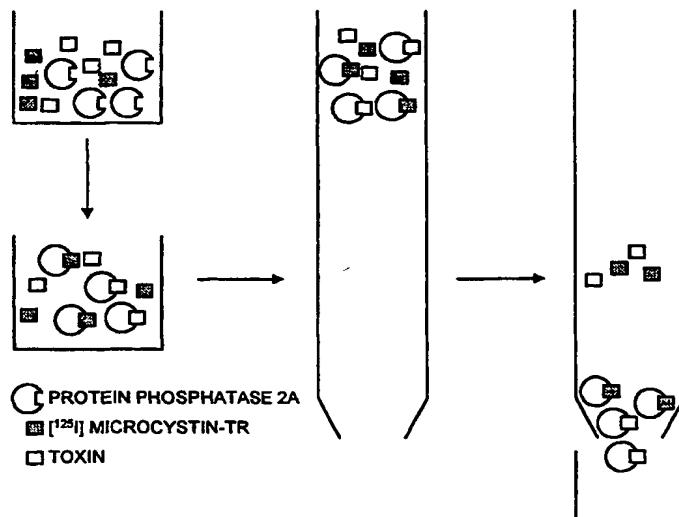
25 19. A kit as claimed in any one of claims 15 to 17 wherein said kit comprises magnetically displaceable polymer micro spheres having immobilized thereon a protein phosphatase;
gold sol labelled okadaic acid molecules capable of competitively inhibiting algal toxins binding to said protein phosphatase.

30 20. Use of the kit as claimed in any one of claims 15 to 19 for the determination of phosphatase-targeting toxins.

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(54) Title: ASSAY FOR PHOSPHATASE-TARGETING TOXINS**(57) Abstract**

The invention provides an assay method for determining phosphatase targeting toxins which inhibit protein phosphatases comprising contacting a solid support having an immobilized ligand immobilized thereon with: (i) a sample suspected of being contaminated with toxin and (ii) a non-immobilized ligand, wherein said immobilized ligand is capable of generating directly or indirectly detectable signal when uncomplexed, when complexed by said toxin, when complexed by a complex of said toxin and said non-immobilized ligand or when complexed by said non-immobilized ligand or said non-immobilized ligand is capable of generating a directly or indirectly detectable signal when uncomplexed or when complexed, separating a bound fraction from a non-bound fraction; and directly or indirectly determining the non-immobilized ligand bound to the immobilized ligand (the bound fraction) or non-complexed in aqueous solution (the non-bound fraction).

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Assay for phosphatase-targetting toxins

The present invention relates to an assay method
5 for the detection of phosphatase-targetting toxins
typically produced by microalgae such as for example
cyanobacteria and dinoflagellates.

Dinoflagellates are typically unicellular,
photosynthetic, bi-flagellated algae. Some of the
10 marine dinoflagellates (e.g. *Prorocentrum* sp. and
Dinophysis sp.) produce phosphatase-targetting toxins
such as okadaic acid and dinophysitoxin, which cause
gastrointestinal problems if ingested by humans. Such
15 algae can thus be problematic if they contaminate the
habitats of shellfish for consumption.

Cyanobacteria, which are often referred to as blue-
green algae, are also photosynthetic organisms which are
principally aquatic and inhabit coastal waters, open sea
20 and oceans, rivers, lakes and ground water but may also
be terrestrial and found in leaf litter and soil.

Many species and strains of cyanobacteria, in
particular *Microcystis* sp., *Aphanizomenon* sp., *Anabena*
sp., *Nodularia* sp. and *Oscillatoria* sp., produce toxins
25 which if ingested by humans or other mammals, birds and
even fish, can produce illness. Ingestion of such
toxins occurs by two main routes, either by drinking
contaminated water or by eating contaminated seafood.

Two particular types of toxins are produced by
cyanobacteria and dinoflagellates. Neurotoxins, for
30 example anatoxins and saxitoxins, cause paralysis in the
victim and hence the condition often referred to as
paralytic shellfish poisoning. Poisoning by such
neurotoxins is rare but can prove to be fatal.

The other form of toxins inactivate protein
35 phosphatase enzymes in the cells of the body by binding
to the enzymes and affecting their ability to
dephosphorylate protein substrates. These toxins are

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relatively common, and some (such as the dinoflagellate toxins okadaic acid and dinophysitis toxin) can cause nausea, vomiting and diarrhoea and hence the condition often referred to as diarrhoeic shellfish poisoning.

5 Some protein phosphatase-targeting toxins are tumour promoters and exposure to these toxins may lead to cancer. Others, such as the cyanobacterial toxins microcystin and nodularin are hepatotoxic and cause liver damage. The most prevalent of the phosphatase targeting toxins are microcystin, nodularin and okadaic acid.

10

The most common sources of dinoflagellate toxin poisoning are shellfish and fish liver, and the most common cause of cyanobacterial toxin poisoning is contaminated drinking and/or bathing water. Both cyanobacterial and dinoflagellate toxins may however be harboured in shellfish and in water. A particularly common source of algal toxin poisoning is mussels since they accumulate the toxins upon feeding on toxin-producing algae. Other shellfish, for example oysters, clams and scallops can also be affected.

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Additionally, domestic water supplies, particularly if they originate from ground water, can become contaminated with cyanobacteria and thus provide a direct route for toxin ingestion.

25

There is some concern regarding consumption of algae and cyanobacteria as a high-protein health food and diet aid. There are no official guidelines for monitoring collected algae or cyanobacteria for contamination by toxin producing strains and the marketing of genera such as *Anabaena* and *Aphanizomenon* is particularly worrying since a number of toxin producing strains may be found within them.

30

In addition to the short term discomfort, medical costs, commercial costs to the shellfish industry, loss of working hours etc. which result from exposure to

35

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algal toxins, as mentioned above the phosphatase targeting toxins microcystin and nodularin have been found to be tumour promoters and it is believed that repeated exposure to such toxins at the clinical or sub-clinical level, particularly in combination with a high intake of alcohol or smoking may result in cancer, especially of the liver.

Presently, a number of different methods exist for the detection and quantitation of phosphatase targeting toxins, from algae and cyanobacteria. One standard method involves grinding mussels or other potential sources of the phosphatase targeting toxins and injecting an extract of the ground mussel tissue into mice. The presence and level of phosphatase-targeting toxin contamination is then determined in relation to mouse survival (Stabell *et al.* (1992), *Food. Chem. Toxicol.* 30(2): 139-44). Clearly, this is a time consuming, crude and expensive method of assessing food safety and quality control.

Another method involves measuring the reduction in enzymic activity of exogenously added phosphatase thus detecting the presence of phosphatase targeting toxins in the shellfish. Again this involves grinding mussels or other shellfish tissue, releasing endogenous phosphatases which interfere with the added phosphatase, compromising the sensitivity and accuracy of the test (Sim and Mudge (1994) in *Detection Methods for Cyanobacterial Toxins* Eds. Codd, Jeffries, Keevil and Potter, Royal Society of Chemistry).

A great need exists therefore for a quick, sensitive, and inexpensive assay or method to allow the qualitative and/or quantitative determination of the presence of phosphatase-targeting toxins, in particular algal and cyanobacterial phosphatase-targeting toxins, in water, shellfish and/or edible products of algae or cyanobacteria. In particular, there is a need for an assay method which is simple enough to be performed on

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site by relatively non-skilled or non-skilled personnel, for example fishmongers or water sanitation personnel and requires no laboratory equipment or special facilities for its performance.

5 Thus, according to a first aspect, the present invention provides an assay method for determining phosphatase targeting toxins which inhibit protein phosphatases comprising contacting a solid support having an immobilized ligand thereon with:

10 (i) a sample suspected of being contaminated with toxin and

(ii) a non-immobilized ligand,

15 wherein said immobilized ligand is capable of binding to at least one of said toxins, to said non-immobilized ligand or to complexes of said toxin and said non-immobilized ligand, and said non-immobilized ligand is capable of binding to at least one of said immobilized ligand, to said toxin or to complexes of said toxin and said immobilized ligand whereby the proportion of said immobilized ligand bound by said toxin, said non-immobilized ligand or complexes of said toxin and said non-immobilized ligand is dependent on the toxin content of said sample and

20 wherein said immobilized ligand is capable of generating directly or indirectly a detectable signal when uncomplexed, when complexed by said toxin, when complexed by a complex of said toxin and said non-immobilized ligand or when complexed by said non-immobilized ligand or said non-immobilized ligand is capable of generating a directly or indirectly detectable signal when uncomplexed or when complexed, separating a bound fraction from a non-bound fraction; and

25 directly or indirectly determining the non-immobilized ligand bound to the immobilized ligand (the bound fraction) or non-complexed in aqueous solution (the non-bound fraction);

- 5 -

wherein the application of (i) and (ii) to the solid support may be performed separately, sequentially or simultaneously and if separately or sequentially, they can be performed in either order.

5 Thus in one embodiment toxin determination may involve determination of the non-immobilized ligand which has failed to bind directly or indirectly to the immobilized ligand. Where the non-immobilized ligand competes for binding to the immobilized ligand with the
10 toxin a high level of unbound ligand is indicative of a high toxin concentration. Where the non-immobilized ligand can complex toxin bound to the immobilized ligand then a high level of unbound ligand is indicative of a low level of toxin concentration.

15 In another embodiment, toxin determination involves determination of the non-immobilized ligand which has bound directly or indirectly to the immobilized ligand. Where toxin and non-immobilized ligand compete for binding to the immobilized ligand then a high level of bound ligand is indicative of a low level of toxin
20 concentration. Where the non-immobilized ligand can complex toxin bound to the immobilized ligand then a high level of bound ligand is indicative of a high level of toxin concentration.

25 Preferably however the method of the invention involves a competitive binding assay for the detection of phosphatase-targeting toxins, in particular algal and cyanobacterial toxins, wherein toxin molecules present in a sample compete with the non-immobilized ligand for
30 a limited number of binding sites of the immobilized ligand and any toxin present in said sample is determined relative to the extent of non-immobilized ligand bound to or not bound to the binding sites of the immobilized ligand.

35 As used herein, the terms "detecting" "determining" or "assessing" include both quantitation in the sense of obtaining an absolute value for the

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amount or concentration of phosphatase-targeting toxins, present in the sample and also semi-quantitative and qualitative assessment or determination. An index, ratio, percentage or molar indication of the level or 5 amount of toxin present may be determined or alternatively a simple indication of presence or absence of such toxins in the sample, may be obtained. In a preferred aspect of the invention a simple presence or absence or semi-quantitative determination of toxin 10 presence is achieved. In this regard "absence" of toxin may mean that the toxin concentration is below the detection limit of the assay or is below a level deemed to be safe or tolerable.

The samples used in the assay method of the 15 invention may be any sample suspected of exposure to phosphatase-targeting toxins, perhaps by exposure to phosphatase-targeting toxin producing microorganisms, for example water which may be sea water, fresh water, ground water, water taken from lakes, rivers, wells, 20 streams, reservoirs, domestic water supplies or may be moisture extracted from shellfish for example by simple draining or extraction using a pipette or water in which shellfish have been allowed to soak or may be a foodstuff, food additive, nutritional supplement, 25 alternative remedy or similar product which is produced by or from algae or cyanobacteria. Where shellfish contain free water (e.g. as in oysters), the assay may involve dipping an absorbent substrate (the solid support) into that water. Alternatively it may simply 30 involve pressing an absorbent substrate against the damp flesh of the shellfish, e.g. after breaking on opening the shell.

In a preferred aspect of the invention the sample under investigation is surface or free moisture from 35 shellfish.

All types of shellfish, for example scallops, prawns, mussels, and oysters are susceptible to the

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assay method of the invention but in a preferred aspect, the shellfish are mussels. In another preferred aspect, the sample under investigation is water taken from the habitat in which such shellfish live and in a further preferred aspect, the sample is water taken from domestic water supplies.

The sample used for analysis may be used in an essentially untreated manner but may optionally be filtered by any known method or diluted by adding water, buffer or any other aqueous medium prior to analysis and may be stored or preserved for example by chilling or freezing prior to analysis.

Any toxin binding ligand may be used in the method of the invention as the immobilized or non-immobilized ligand for example antibodies, which may be polyclonal or monoclonal, or antibody fragments for example F(ab), F(ab')₂ or F(v) fragments. Such antibodies or antibody fragments may be monovalent or divalent and may be produced by hybridoma technology or be of synthetic origin, either as products of recombinant DNA technology or chemical synthesis. Single chain antibodies or other antibody derivatives or mimics could for example be used. The antibodies or antibody fragments may be directed or raised against any epitope, component or structure of the phosphatase-targeting toxins as appropriate. Alternatively, compounds with an affinity for the toxin for example a small organic molecule or peptide, e.g. an oligopeptide or polypeptide, capable of specifically binding the toxin for example a specific binder selected from a combinatorial chemistry or phage display library or a specifically binding sequence of DNA or RNA could be used.

Preferably however, the toxin binding ligand of the present invention is a protein phosphatase enzyme, and even more preferably the binding ligand protein phosphatase 2A (pp2A) is used in the assay method.

Likewise, the second ligand used in the method of

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the invention may be any ligand which binds to the toxin either competitively or non competitively with the first ligand. Alternatively, the second ligand may be any ligand which competes with the toxin for binding to the 5 first ligand. Preferably the first ligand is a toxin binding ligand, more preferably a protein phosphatase enzyme. One of the two ligands must be immobilized and the other must be non-immobilized and one of the ligands must be directly or indirectly detectable. In a 10 preferred embodiment the non-immobilized ligand should meet the functional requirements that it competitively inhibits toxin binding to the immobilized ligand and can directly or indirectly produce a detectable signal, e.g. it may be a molecule which can be labelled using a 15 direct or indirect signal forming moiety of any known form. Such ligands may likewise take the form of antibodies, which may be polyclonal or monoclonal, or antibody fragments for example F(ab), F(ab')₂ or F(b) fragments. Such antibodies or antibody fragments may be 20 monovalent or divalent and may be produced by hybridoma technology or be of synthetic origin, either recombinant DNA technology or chemical synthesis. Single chain antibodies or other antibody derivatives or mimics and small organic molecules, peptides, oligopeptides and 25 polypeptides selected from combinatorial or phage display libraries, could for example be used. The antibodies or antibody fragments may be directed or raised against any epitope, component or structure of the phosphatase-targeting toxin molecule or the ligand 30 which binds the phosphatase targeting molecule as appropriate. Alternatively, compounds with an affinity for the toxin or for the ligand which binds the toxin, for example a small organic molecule or peptide, oligopeptide or polypeptide capable of specifically 35 binding the toxin or the ligand which binds the toxin , for example a specific binder selected from a

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combinatorial chemistry or phage display library, or a specifically binding sequence of DNA or RNA could be used.

The reporter moiety which one of the ligands will generally carry may be a binding site for a directly detectable moiety, e.g. a metal sol (e.g. gold sol), a chromophore or fluorophore (e.g. a cyanine, phthalocyanine, merocyanine, triphenylmethyl, equinace, etc. see Topics in Applied Chemistry, Infrared Absorbing Chromophores, edited by M. Matsuoka, Plenum Press, New York, NY, 1990, Topics in Applied Chemistry, The Chemistry and Application of Dyes, Waring et al. Plenum Press, New York, NY, 1990, and Handbook of Fluorescent Probes and Research Chemicals, Haugland, Molecular Probes Inc. 1996, a radiolabel, an enzyme, a magnetic particle, a turbidity inducing agent, etc., or it may already carry such a directly detectable moiety. Where the reporter moiety is carried by the immobilized ligand it will generally be a binding site for a directly detectable moiety which binding site is either activated, or more generally deactivated, when the ligand is complexed.

Preferably the reporter moiety is carried by the non-immobilized ligand.

In a preferred embodiment of the invention, the non-immobilized ligand is a labelled, e.g. enzyme or chromophore or fluorophore labelled peptide hepatotoxin, e.g. a hepatotoxin selected from nodularin, microcystin LC or microcystin YR or alternatively okadaic acid.

While labelling with radiolabels is possible, since the assay is primarily intended for on-site use by lay users, it is preferable to use reporter moieties that give a visible signal, e.g. chromophores, fluorophores, phosphorescent moieties, turbidity inducing agents, gas evolution inducing agents, etc.

Where the signal forming moiety is a material which

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binds to a binding site on one of the ligands, it will conveniently be contacted with the bound or unbound fraction, as appropriate, after separation of the bound and unbound fractions.

5 In general, where the signal is to be derived from the bound fraction, it will be preferable to rinse the substrate, e.g. with water, to flush away the unbound fraction before the ligand is detected or generated and detected.

10 Any species or strain of algae or cyanobacteria which produces phosphatase-targeting toxins may be subject to the present invention but it is particularly applicable to toxin producing strains of cyanobacteria for example *Microcystis aeruginosa*, *Anabena* species, 15 *Nodularia spumigena* and *Anabaena flos-aquae* or algae. Thus for example the toxins microcystin-LR and microcystin-YR are produced by *Microcystis* sp., the toxin nodularin is produced by *Nodularia* sp. and the toxin okadaic acid is produced by *Prorocentrum* sp.

20 The toxins subject to determination by the present method may likewise be any phosphatase-targeting toxin produced by algae or cyanobacteria, but in preferred aspects the peptide toxins are hepatotoxins (of which microcystin and nodularin are the most prevalent) or 25 okadaic acid.

Thus, in its most general sense, the method of the invention involves simply contacting a sample suspected of contamination with phosphatase-targeting toxins, with a toxin binding ligand and a reporter molecule capable 30 of competing with said toxin for the binding sites of the ligands either simultaneously, sequentially or separately in either order, the reporter molecule optionally being bound to the binding ligand prior to exposure to the sample under investigation, and 35 determining the reporter molecule which is either bound to the solid phase or free in solution.

The bound fraction may be separated from the unbound

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faction prior to assessment of reporter by any suitable means, for example, precipitation, centrifugation, filtration, chromatographic means, capillary action or simply by draining. The solid phase may for example be
5 in the form of a dipstick or a solid matrix in any known form for example polymeric or magnetic beads for example Dynabeads® (available from Dynal AS). In preferred embodiments of the present invention, the solid phase to which the toxin binding ligands are immobilised is in
10 the form of Dynabeads®.

The reporter molecule may be assessed in either the bound or the non-bound fraction depending on the specific embodiment of the invention but preferably it is assessed in the bound fraction.

15 The immobilized ligand may be immobilised by any known means, for example by binding or coupling the ligand to any of the well known solid supports or matrices which are currently widely used or proposed for separation or immobilisation for example solid phases
20 may take the form of particles, sheets, gels, filters, membranes, fibres or capillaries or microtitre strips, tubes or plates of wells etc. and conveniently may be made of glass, silica, latex, a polymeric material or magnetic beads. Techniques for binding the ligand to
25 the solid support are well known in the art and widely described in the literature. In preferred embodiments of the present invention, the solid phase to which the phosphatase-targeting toxin binding ligands are immobilised is in the form of Dynabeads®.

30 The assay method of the present invention is advantageous in that it can be performed without the need of complex laboratory equipment and can be performed by the relatively non-skilled or non-skilled person. Hence, the assay method is suitable for use in
35 the home, in shops or in the field and it can be performed quickly and easily without the need for intensive labour or hazardous chemicals.

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Of particular advantage in the assay of the present invention is the very high degree of sensitivity which is of critical importance when analysing samples wherein the toxin is present at very low levels for example in
5 the testing of drinking water or assessing possible pollution with phosphatase targeting toxins. Typically the assay is capable of detecting toxins in picomolar concentrations, e.g. as low as 10 pM. Conveniently the assay may be used to detect toxins in the 15 to 560 pM
10 range.

A further advantage of the present assay relative to existing techniques is that the present assay is not affected by the presence of endogenous phosphatases which may be present in the samples under analysis,
15 particularly, for example, if the samples are taken from shellfish.

In one embodiment of the present invention, a protein phosphatase is immobilised on a solid support, the immobilised phosphatase is contacted with the sample under investigation and any phosphatase-targeting toxin present in the sample binds to the immobilised phosphatase. A source of reporter molecules which compete with the toxin for phosphatase binding sites is added. The reporter molecules displace toxin molecules from the binding sites to a degree which depends upon the relative concentration of toxin molecules and reporter molecules. The degree of reporter molecule binding facilitates determination of toxin present in the sample under investigation. Preferred reporters/labels include radiolabels, chromophores (including fluorophores) and enzymes which give rise to chromogenic or fluorogenic products. Scintillation proximity labels and labels which give rise to a measurable change in light scattering are also to be considered.
25
30

35 In an alternative embodiment, solid support immobilised reporter-blocked phosphatase molecules are contacted with the sample under investigation and any

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phosphatase-targeting toxins present in the sample compete with the phosphatase bound reporter molecules displacing them from the solid phase into the aqueous phase in a degree proportional to the amount of toxin 5 present in the sample. The amount of reporter molecule which remains bound to the solid phase is then assessed to facilitate determination of toxin presence in the sample under investigation.

Viewed from a further aspect, the invention 10 provides a kit for the detection of cyanobacterial or algal phosphatase-targeting toxins, according to the invention, said kit comprising:

a solid phase upon which is immobilised a ligand; 15 a non-immobilized ligand, preferably in aqueous solution or complexed to the immobilized ligand; where neither of said immobilized and non-immobilized ligands includes a directly or indirectly detectable moiety, a reporter moiety capable of binding to one of said immobilized and non-immobilized ligands and generating a 20 detectable signal, preferably said detectable moiety or signal being directly readable without laboratory equipment.

In one preferred embodiment, the kit of the present invention comprises:

25 a solid phase upon which is immobilized phosphatase-targeting toxin binding ligands; a reporter molecule capable of competitively inhibiting binding of phosphatase-targeting toxins to 30 said toxin binding ligand and generating a signal readable without laboratory equipment.

An especially preferred embodiment of the kit of the invention comprises magnetically displaceable polymer micro spheres having immobilized thereon a protein phosphatase;

35 gold sol labelled peptide hepatotoxin molecules capable of competitively inhibiting cyanobacterial toxins binding to said protein phosphatase.

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A further especially preferred embodiment of the kit of the invention comprises magnetically displaceable polymer micro spheres having immobilized thereon a protein phosphatase;

5 gold sol labelled okadaic acid molecules capable of competitively inhibiting algal toxins binding to said protein phosphatase.

In another preferred aspect, use of the kit involves dipping a porous cellulosic substrate on which 10 a toxin binding ligand is immobilized and which is impregnated with a competitively binding, chromophore (or fluorophore etc) labelled ligand into a sample of water or shellfish fluid, allowing the saturated substrate to incubate for a pre-set period (either 15 removed from the sample or in a pre-set volume of the sample), removing non-bound labelled ligand, e.g. by flushing the substrate with toxin-free water or by leaving the substrate to soak for a pre-set period in a pre-set volume of toxin free water, and inspecting the 20 colour of the substrate or of the soaking water. Desirably, the substrate is mounted on a support, preferably one marked with calibration colours to facilitate comparison of the substrate or soaking water colour to determine toxin concentration or to indicate 25 whether toxin concentration is above or below one or more threshold values.

The invention will now be illustrated by the following non-limiting examples:

30 **Materials**

Microcystin YR, Microcystin-LR, okadaic acid, nodularin, calyculin A and tautomycin are purchased from Calbiochem (San Diego, CA). Carrier-free Na¹²⁵I and [γ -³²P]ATP is obtained from Amersham (Little Chalfont, UK). Albumin (RIA grade), ammonium acetate, Chloramine T, dimethyl sulfoxide (DMSO), dithioerythritol (DTE), EDTA, EGTA,

- 15 -

glycerol, Hepes, histone II-AS, sodium metabisulfite and trypsin inhibitor (soybean) are purchased from Sigma (St Louis, MO). Acetonitrile and trifluoroacetic acid (TFA) are purchased from Rathburn (Walkerburn, Scotland).

5 Partially purified protein phosphatase 2A is either purchased from Upstate Biotechnology (Lake Placid, NY) or purified according to Resink et al. (Eur. J. Biochem. 133: 455-461 (1983)).

10 Iodination of microcystin-YR

Microcystin YR (10 µg) is iodinated with 1 mCi carrier-free Na¹²⁵I (37 MBq) using chloramine T as described by Ciechanover et al., (PNAS 77: 1365-1368 (1980)).

15 Following the iodination reaction, iodide is separated from [¹²⁵I]microcystin-YR using Sep-Pak® Plus cartridges (Waters, Milford, MA) according to the method of Runnegar et al. (Toxicon 24: 506-509 (1986)). The [¹²⁵I]microcystin-YR is applied to a 3x250 mm Inertsil ODS-2 HPLC column from Chrompack (Raritan, NJ) and eluted with an acetonitrile gradient.

Competitive binding assay

25 The competitive binding assay is carried out in a volume of 0.5 ml buffered with 50 mM Hepes (pH 7.2), 1 mM EDTA, 0.3 mM EGTA, 1 mM DTE, 5 mM MnCl₂, 0.5 mg ml⁻¹ BSA, and 0.2 mg ml⁻¹ trypsin inhibitor. Algal toxins diluted in 100% DMSO are added to the assay at 0-100 nM in a final concentration of 10% DMSO. [¹²⁵I]microcystin-YR (1 Ci/13 ng) is added at 35 pM. Protein phosphatase 2A (30 pM) is added last, and the reaction mixture is incubated on ice overnight. [¹²⁵I]microcystin-YR bound to protein phosphatase 2A is separated from free [¹²⁵I]microcystin-YR by gel filtration using Sephadex® G-50 fine from Pharmacia (Uppsala, Sweden) in 0.7 x 15 cm columns from Bio-Rad (Hercules, CA). A 50 mM Hepes buffer (pH 7.2)

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with 1 mM EDTA and 0.3 mM EGTA is used in the separation which is done at 4°C. The fraction containing [¹²⁵I]microcystin-YR which binds to protein phosphatase 2A is collected and the radioactivity is quantitated by scintillation counting. Nonspecific binding of [¹²⁵I]microcystin-YR is detected in a control reaction where microcystin-LR is added at an excess (1 μM).

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Example 1

Protein phosphatase 2A is coupled to magnetic beads (directly to the beads or via biotinylation of the phosphatase). Immobilized protein phosphatase is then 5 mixed with sample and radiolabelled toxin (e.g. [¹²⁵I]-microcystin-YR). The immobilized protein phosphatase is separated from the reaction mixture by magnetic force. Radioactivity associated with the protein phosphatase (magnetic bead) is detected by scintillation counting. 10 The amount of radiolabel associated with the protein phosphatase decreases as a function of phosphatase binding toxin in the sample.

Example 2

15 Protein phosphatase 2A is coupled to magnetic beads (directly to the beads or via biotinylation of the phosphatase). Immobilized protein phosphatase is then mixed with sample and toxin coupled to colored beads. The immobilized protein phosphatase is separated from 20 the reaction mixture by magnetic force. Colored beads associated with the protein phosphatase (magnetic beads) are evaluated by eye or by a low magnification microscope (e.g. Nikon TMS). The amount of colored beads associated with the protein phosphatase (magnetic 25 beads) decreases as a function of phosphatase binding toxin in the sample.

Example 3

30 Protein phosphatase 2A is coupled to magnetic beads (directly to the beads or via biotinylation of the phosphatase). Immobilized protein phosphatase is then mixed with sample and toxin immobilized on beads carrying an immobilized enzyme. The enzyme is capable 35 of producing a detectable product (colored or fluorescent) upon appropriate incubation with a chromogenic or fluorogenic substrate. The immobilized protein phosphatase is separated from the reaction

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mixture by magnetic force. Color or fluorescence associated with the protein phosphatase (magnetic beads) is measured by spectroscopy or fluorimetry, respectively. The amount of color/fluorescence 5 associated with the magnetic beads decreases as a function of phosphatase binding toxin in the sample.

Example 4

Scintillation Proximity Assay:

10 Protein phosphatase is biotinylated and immobilized to wells precoated with streptavidin and a scintillant (e.g. FlashPlate PLUS Streptavidin SMP103 supplied by NEN). The sample and [¹²⁵I]microcystin-YR are added to the wells. The amount of [¹²⁵I]microcystin-YR bound to 15 the immobilized protein phosphatase is detected by scintillation counting.

Example 5

20 Inhibition of binding of [¹²⁵I]-microcystin-YR to protein phosphatase 2A in the presence of various toxins

Compound tested ¹	IC ₅₀ ² (pM)
nodularin	15
microcystin-LR	17
microcystin-YR	75
okadaic acid	100
calyculin A	251
tautomycin	562

1 The compounds tested were incubated with [¹²⁵I]-microcystin-YR and protein phosphatase 2A as described above.

2 The IC₅₀ value represents the concentration needed to obtain a 50% inhibition of [¹²⁵I]-microcystin-YR

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binding to protein phosphatase 2A. These values were determined according to Fig. 3. The data represent an average of at least 3 separate experiments.

5 Example 6

Effect of exogenous compounds on the competitive binding assay as compared to the protein phosphatase assay

10	Compound tested ¹	% activity ²	
		Competitive binding assay	Protein phosphatase assay
15	2 mM ATP	103.3 ± 0.2	9.8 ± 3.4
	0.5 mM ATP	101.6 ± 1.7	29.8 ± 5.6
	0.05 mM NaPPi	101.4 ± 4.1	14.2 ± 1.2
	50 mM NaF	101.5 ± 1.9	7.7 ± 1.4
	5 mM NaF	102.0 ± 3.3	62.6 ± 0.4
20	1 mg/ml caseine	98.6 ± 4.5	3.4 ± 0.2
	0.02 mg/ml caseine	98.9 ± 6.1	33.3 ± 4.9
	5 mg/ml histone 2A	91.9 ± 1.8	1.4 ± 0.1
	0.002 mg/ml histone	95.2 ± 4.7	63.6 ± 4.0
	0.5 M NaCl	41.2 ± 0.7	44.4 ± 1.6
25	seawater	34.8 ± 0.4	ND
	10% seawater	87.3 ± 0.4	ND
	10% DMSO	72.8 ± 2.3	97.9 ± 3.3
	10% MeOH	73.9 ± 0.5	87.4 ± 4.1
	10% acetonitrile	90.4 ± 5.4	88.2 ± 2.7
30	0.4% Triton X-100	122.3 ± 1.0	60.2 ± 5.7
	0.4% Nonidet P-40	106.0 ± 2.0	61.1 ± 1.3
	0.4% CHAPS	90.9 ± 9.9	138.0 ± 34.4

1 Protein phosphatase 2A was preincubated with the
35 compounds dissolved in 50 mM Hepes (pH 7.2) or with
 buffer alone (control) for 30 minutes on ice.
 Phosphatase activity was measured by
 dephosphorylation of phosphohistone as described.
 The % activity is relative to the control reaction.

40 2 The activity in the competitive binding assay
 represents the ability of protein phosphatase 2A to

- 20 -

bind [¹²⁵I]microcystin-YR in the presence of the exogenous compound dissolved in buffer relative to buffer alone. The data represents an average of at least three separate experiments ± SEM.

Example 7

Sensitivity of the binding assay for nodularin and microcystin-LR

5

inhibition of [¹²⁵I]microcystin-YR binding (%)¹

toxin	(M)	milliQ water	drinking water	sea water	sea water, 1/10 ²
nodularin	1E-10	88.37 ± 0.31	88.75 ± 0.16	72.18 ± 0.82	67.24 ± 0.66
	5E-11	36.37 ± 2.28	36.12 ± 1.04	48.47 ± 0.79	52.98 ± 1.98
microcystin-LR	1E-10	84.91 ± 0.42	86.97 ± 1.12	73.31 ± 1.20	46.41 ± 5.97
	5E-11	13.87 ± 3.16	12.85 ± 0.88	49.34 ± 3.82	38.61 ± 1.49

¹ Nodularin and microcystin-LR were dissolved in MilliQ, drinking, or sea water at the concentration shown. Aliquots of 300 µl of these solutions were tested for their ability to compete with [¹²⁵I]microcystin-YR for the binding of protein phosphatase 2A as described above.

15

² Sea water diluted 1/10 in milliQ water.

The data is presented as the average ± SEM.

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Example 9

Okadaic acid equivalents in shellfish extracts as determined by HPLC analysis and by the protein phosphatase binding assay

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25

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Extract ¹	OA equivils. by HPLC analysis ²	OA equivils by binding assay ³
	(μ g/g hepatopancreas)	(nM)
1	0	0
2	0	0
3	0	0
4	4	2480
5	1.2	748
6	0.8	496

¹ The extracts were made from hepatopancreas of mussels collected along the Norwegian coast.

² The extracts were analyzed for okadaic acid equivalents by HPLC.

³ The extracts were diluted in 100% DMSO and tested for their ability to compete with [¹²⁵I]microcystin-YR for binding to protein phosphatase 2A using the binding assay as described above. The concentration of okadaic acid equivalents were determined by comparing the data to standard curves of okadaic acid dissolved in 100% DMSO.

Example 9

Attached Diagrams

35

Fig. 1 of the attached diagram is a schematic diagram of the competitive binding assay for the detection of protein phosphatase binding toxins.

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Protein phosphatase 2A is incubated with [¹²⁵I]microcystin-YR and another toxin directed towards protein phosphatase 2A. The toxin competes with the [¹²⁵I]microcystin-YR for binding to the phosphatase.

5 Addition of a large amount of toxin results in a reduced binding of [¹²⁵I]microcystin-YR to the phosphatase and vice versa. After binding equilibrium is reached, the [¹²⁵I]microcystin-YR bound to protein phosphatase 2A is separated from free [¹²⁵I]microcystin-YR by gel

10 filtration chromatography. The fraction containing [¹²⁵I]microcystin-YR bound to the phosphatase is collected and the amount of radioactivity determined by scintillation counting.

15 Fig. 2 of the attached diagrams shows the effect of increasing amounts of different algal toxins on binding of [¹²⁵I]microcystin-YR to protein phosphatase 2A.

Protein phosphatase 2A (30 pM) was incubated in the presence 35pM [¹²⁵I]microcystin-YR (1 Ci/13 ng) and 0-100 nM of different algal toxins indicated in the figure. The [¹²⁵I]microcystin-YR bound to protein phosphatase 2A was isolated by gel filtration chromatography and the radioactivity determined by scintillation counting.

20 Each curve represents the average of at least 3 separate experiments.

Fig. 3 of the attached diagrams shows the IC₅₀ for microcystin-LR binding in the competitive binding assay.

30 Binding of [¹²⁵I]microcystin-YR to protein phosphatase 2A was plotted as the ratio between unbound [¹²⁵I]microcystin-YR (Co-Cx) and bound [¹²⁵I]microcystin-YR (Cx) against the concentration of microcystin-LR. Co

35 represents the amount of bound [¹²⁵I]microcystin-YR in the absence of microcystin-YR, and Cx represents the amount of bound [¹²⁵I]microcystin-YR in the presence of

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various concentrations of microcystin-LR.

Fig. 4 of the attached diagrams illustrates the stability of the [¹²⁵I]microcystin-YR bound to protein phosphatase 2A in the presence of excess microcystin LR.
5

Protein phosphatase 2A (1 nM) was incubated in the presence of [¹²⁵I]microcystin-YR (100 pM) for 1 hour. Microcystin-LR (2 μM) was added to the reaction mixture
10 at time 0. The amount of [¹²⁵I]microcystin-YR bound to protein phosphatase 2A was determined for the indicated timepoints by gel filtration and scintillation counting as described. The curve represents an average of 4 separate experiments.

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Claims:

1. An assay method for determining phosphatase targeting toxins which inhibit protein phosphatases comprising contacting a solid support having an immobilized ligand immobilized thereon with:
 - (i) a sample suspected of being contaminated with toxin and
 - (ii) a non-immobilized ligand,
 - wherein said immobilized ligand is capable of binding to at least one of said toxins, to said non-immobilized ligand or to complexes of said toxin and said non-immobilized ligand, and said non-immobilized ligand is capable of binding to at least one of said immobilized ligand, to said toxin or to complexes of said toxin and said immobilized ligand whereby the proportion of said immobilized ligand bound by said toxin, said non-immobilized ligand or complexes of said toxin and said non-immobilized ligand is dependent on the toxin content of said sample and
 - wherein said immobilized ligand is capable of generating directly or indirectly detectable signal when uncomplexed, when complexed by said toxin, when complexed by a complex of said toxin and said non-immobilized ligand or when complexed by said non-immobilized ligand or said non-immobilized ligand is capable of generating a directly or indirectly detectable signal when uncomplexed or when complexed,
 - separating a bound fraction from a non-bound fraction; and
 - directly or indirectly determining the non-immobilized ligand bound to the immobilized ligand (the bound fraction) or non-complexed in aqueous solution (the non-bound fraction);
 - wherein the application of (i) and (ii) to the solid support may be performed separately, sequentially or simultaneously and if separately or sequentially,

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they can be performed in either order.

2. An assay method as claimed in claim 1 wherein said phosphatase-targeting toxin is produced by algae or by
5 cyanobacteria.
3. An assay method as claimed in either of claims 1 and 2 wherein the toxin to be determined is a heptatoxin or okadaic acid.
10
4. An assay method as claimed in any one of claims 1 to 3 wherein toxin molecules present in the sample compete with the non-immobilized ligand for a limited number of binding sites of the immobilized ligand and
15 any toxin present in said sample is determined relative to the extent of non-immobilized ligand bound to or not bound to the binding sites of the immobilized ligand.
5. An assay method as claimed in any one of claims 1
20 to 4 wherein the presence or absence of a phosphatase-targeting toxin is determined.
6. An assay method as claimed in any one of claims 1 to 5 wherein the sample under investigation is surface
25 or free moisture from shellfish, or water taken from the habitat in which such shellfish live, or water taken from domestic water supplies.
7. An assay method as claimed in any one of claims 1
30 to 6 wherein the immobilized and/or non-immobilized ligand is an antibody or antibody fragment.
8. An assay method as claimed in any one of claims 1 to 7 wherein the toxin binding ligand is a protein
35 phosphatase enzyme.

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9. An assay method as claimed in claim 8 wherein the protein phosphatase enzyme is the binding ligand protein phosphatase 2A.

5 10. An assay method as claimed in any one of claims 1 to 9 wherein either the immobilised or non-immobilised ligands carries a reporter moiety.

10 11. An assay method as claimed in claim 10 wherein the non-immobilized ligand carries a reporter moiety.

12. An assay method as claimed in claim 11 wherein the non-immobilized ligand is a labelled peptide hepatotoxin or labelled okadaic acid.

15 13. An assay method as claimed in claim 12 wherein the hepatotoxin is selected from nodularin, microcystin LC or microcystin YR.

20 14. An assay method as claimed in any one of claims 1 to 13 wherein the solid support is a dipstick or solid matrix.

25 15. An assay method as claimed in claim 14 wherein the solid matrix is polymeric or magnetic beads.

16. A kit for the detection of phosphatase-targeting toxins according to the invention, said kit comprising:

30 a solid phase upon which is immobilised a ligand;
a non-immobilized ligand, preferably in aqueous solution or complexed to the immobilized ligand;
where neither of said immobilized and non-immobilized ligands includes a directly or indirectly detectable moiety, a reporter moiety capable of binding to one of said immobilized and non-immobilized ligands and generating a detectable signal, preferably said detectable moiety or signal being directly readable

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without laboratory equipment.

17. A kit as claimed in claim 16 wherein said phosphatase-targeting toxin is produced by algae or by
5 cyanobacteria.

18. A kit as claimed in either of claims 16 and 17 wherein said kit comprises:

10 a solid phase upon which is immobilized phosphatase-targetting toxin binding ligands;
a reporter molecule capable of competitively inhibiting binding of phosphatase-targetting toxins to said toxin binding ligand and generating a signal readable without laboratory equipment.

15 19. A kit as claimed in any one of claims 16 to 18 wherein said kit comprises magnetically displaceable polymer micro spheres having immobilized thereon a protein phosphatase;

20 gold sol labelled peptide hepatotoxin molecules capable of competitively inhibiting cyanobacterial toxins binding to said protein phosphatase.

25 20. A kit as claimed in any one of claims 16 to 18 wherein said kit comprises magnetically displaceable polymer micro spheres having immobilized thereon a protein phosphatase;

30 gold sol labelled okadaic acid molecules capable of competitively inhibiting algal toxins binding to said protein phosphatase.

21. Use of the kit as claimed in any one of claims 16 to 20 for the determination of phosphatase-targeting toxins.

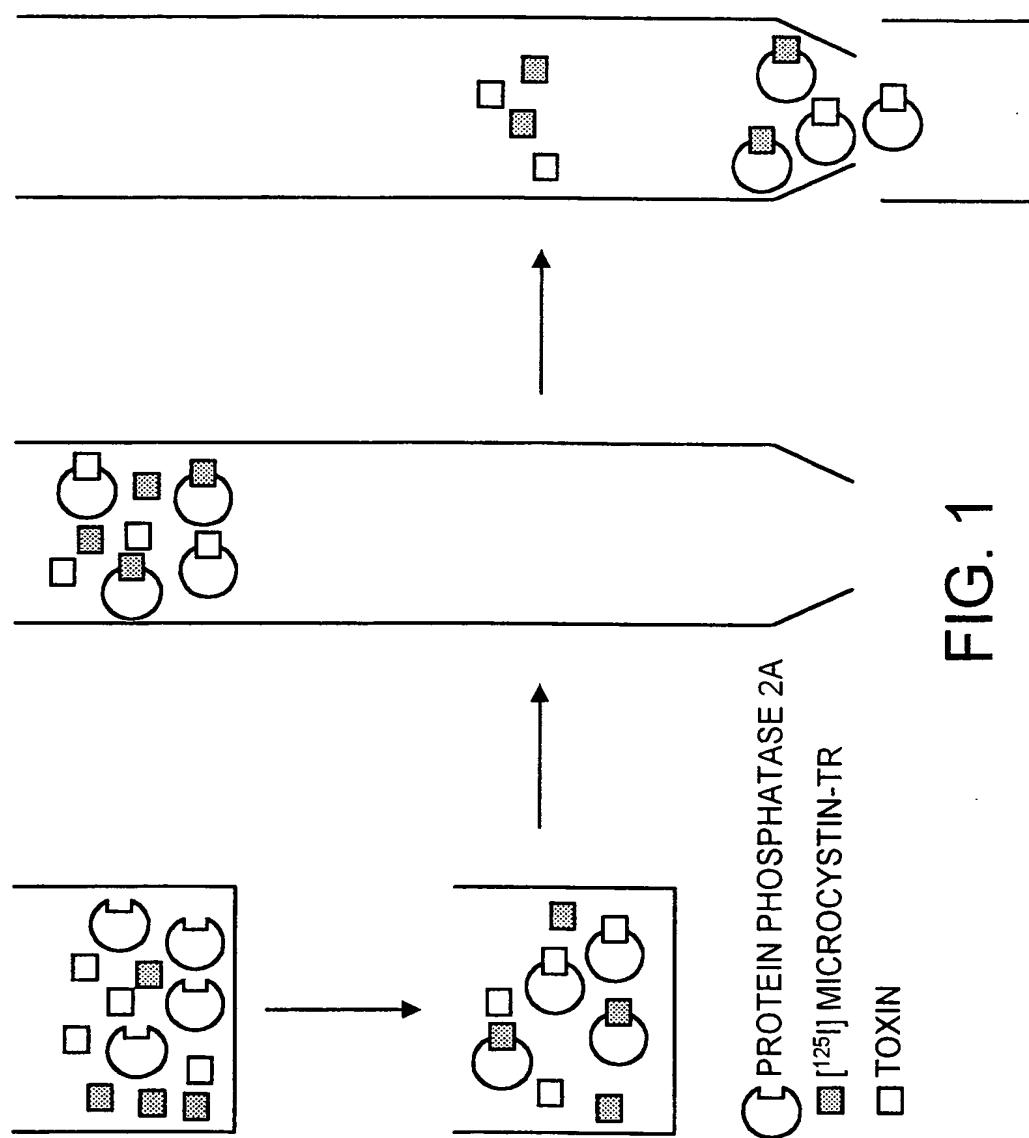
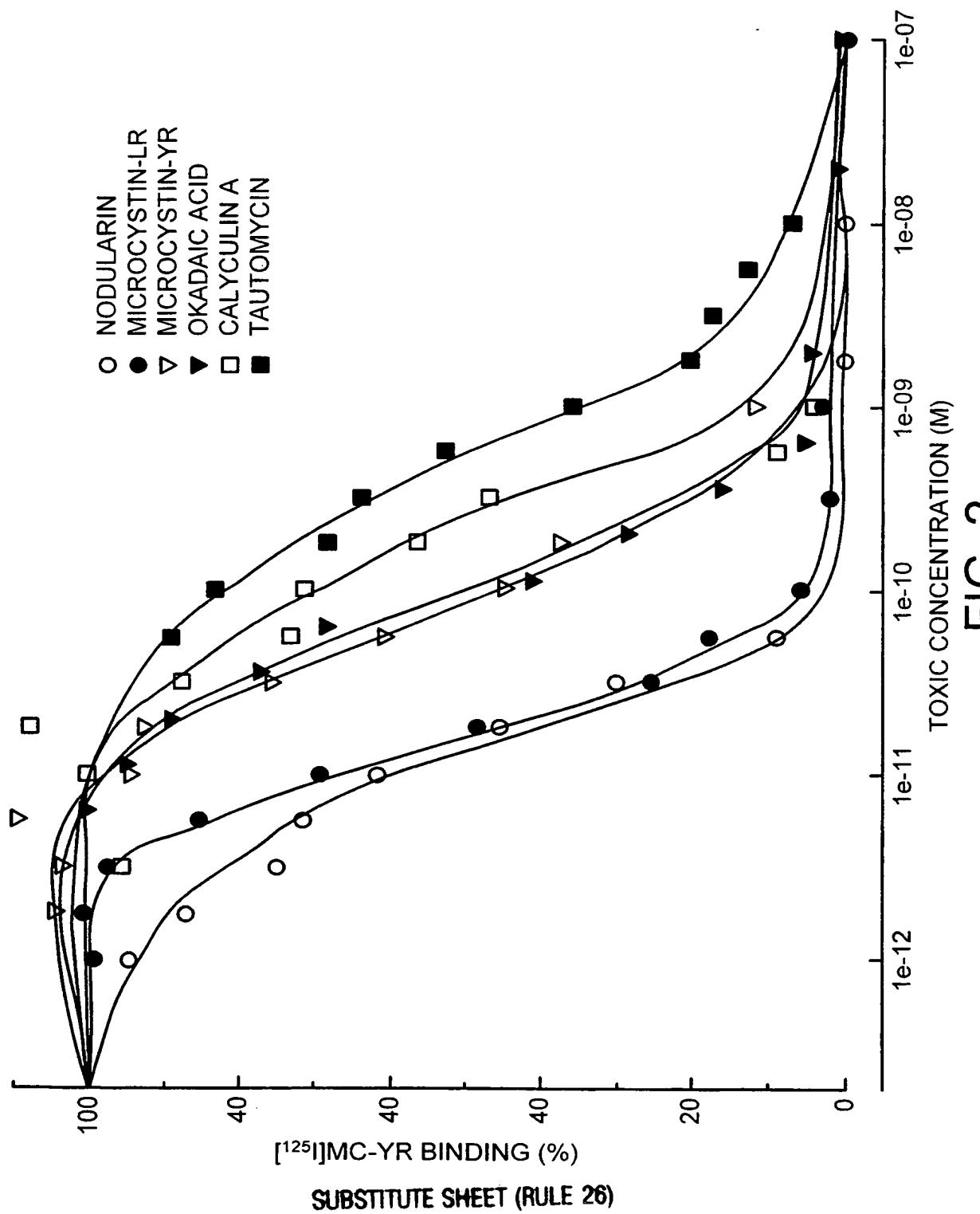


FIG. 1

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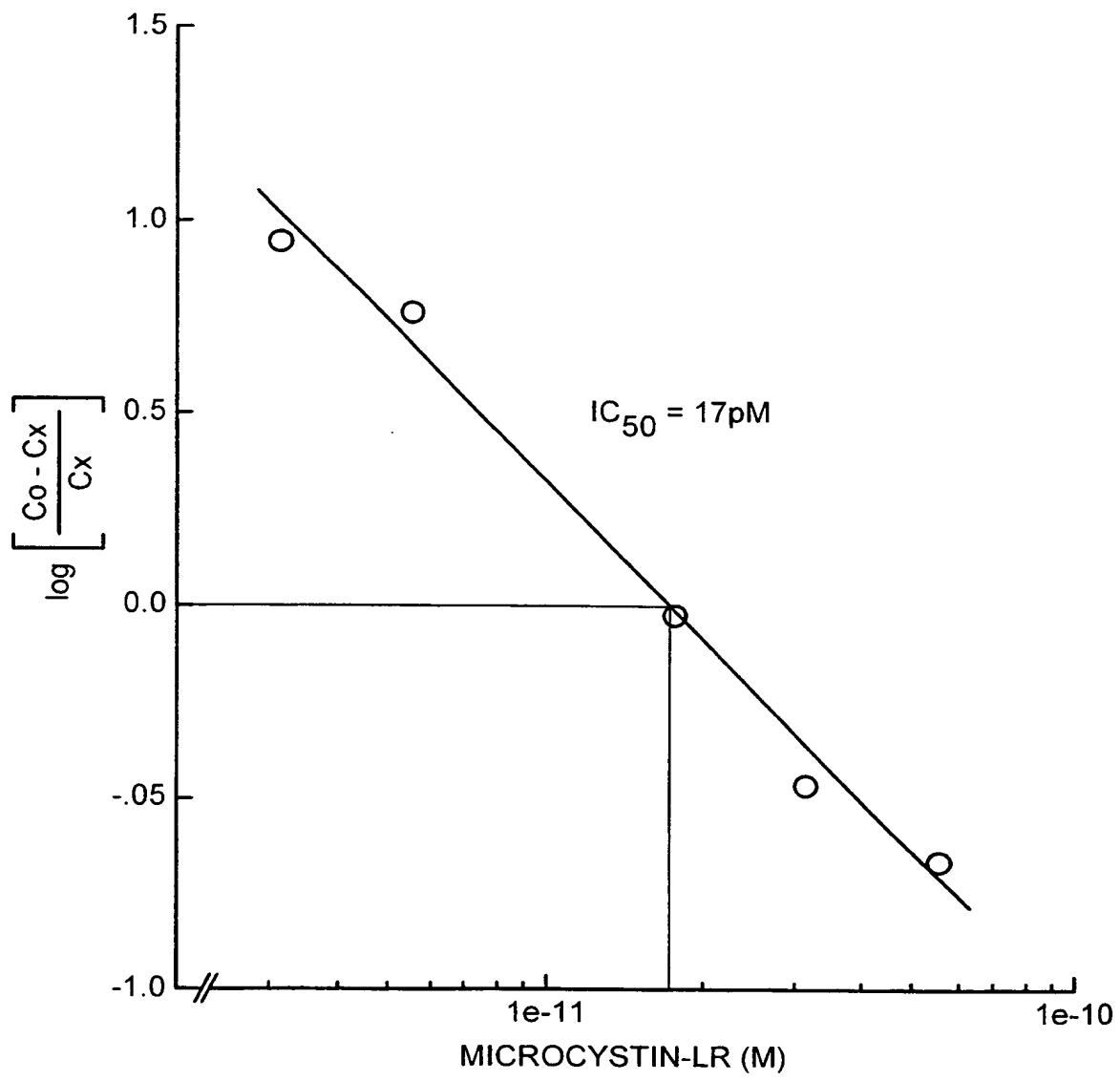
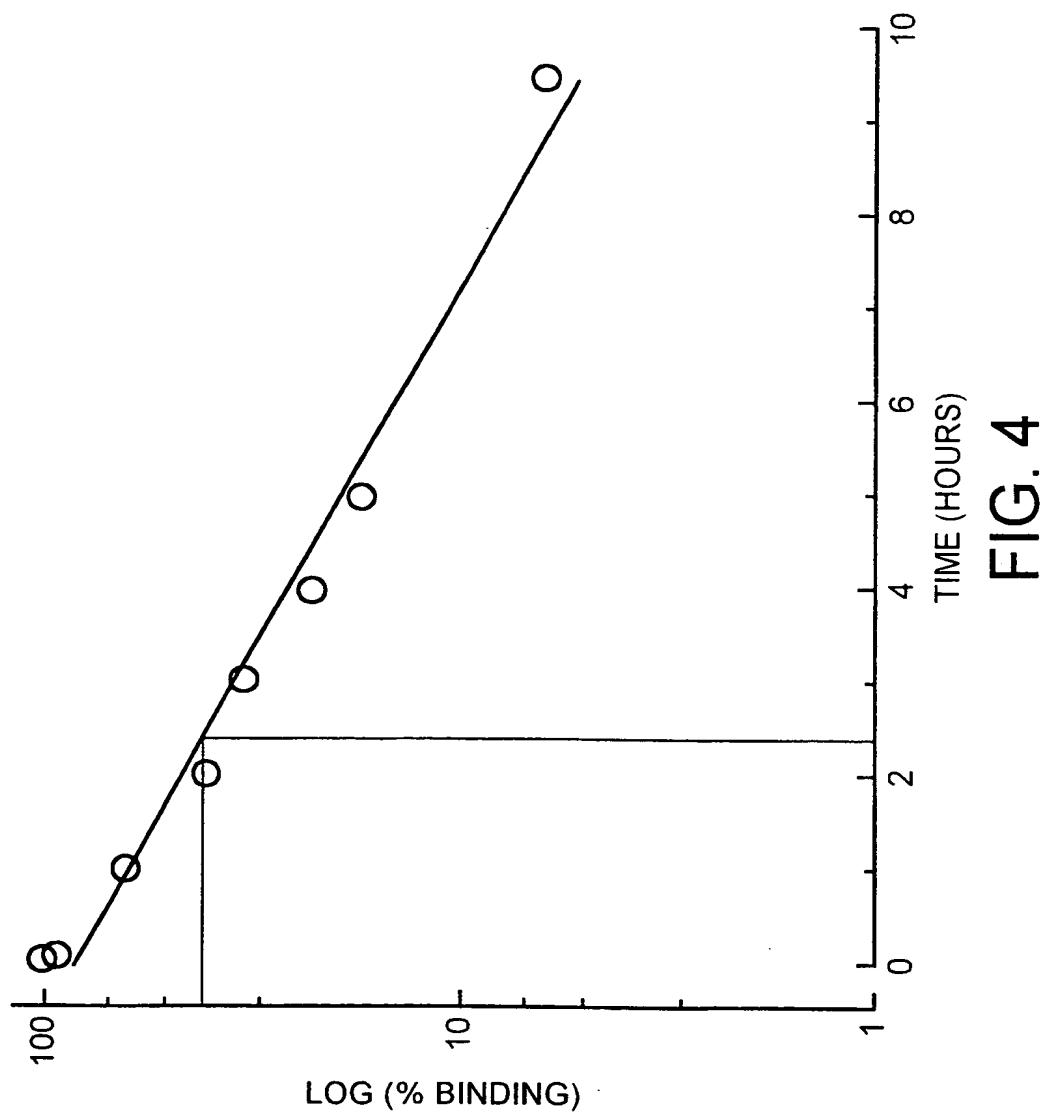


FIG. 3

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03756

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 G01N33/53 C12Q1/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 180 665 A (HOLMES CHARLES) 19 January 1993 (1993-01-19) the whole document ----	1
A	US 5 525 525 A (HOKAMA YOSHITSUGI) 11 June 1996 (1996-06-11) the whole document ----	1,16
A	EP 0 554 458 A (IATRON LAB ;OSAKA PREFECTURE (JP)) 11 August 1993 (1993-08-11) abstract ----	1,16
A	EP 0 311 456 A (UBE INDUSTRIES) 12 April 1989 (1989-04-12) abstract ----	1,16
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

Date of mailing of the international search report

9 February 2000

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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US YANG, MENG SU (1) ET AL: "Effects of microcystins on phosphorylase-a binding to phosphatase-2A: Kinetic analysis by surface plasmon resonance biosensor." retrieved from STN XP002130132 abstract & BIOCHIMICA ET BIOPHYSICA ACTA, (MARCH 14, 1999) VOL. 1427, NO. 1, PP. 62-73. , -----</p>	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

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